Table 1. Quality review of chloroprene.model and supporting files, dated 07/19/19

File or variable	Definition	Notes and determination
Pre-processing steps		
chloroprene.model	Primary model definition file	Commented out "CPUM" since not used in script. CV and CI added to output set.
buildmodel.R	Script is used to generate C code from **chloroprene.model.**	Initially failed because of "file.remove" lines, where the corresponding files were not in the folder. Also the "mPath" variable is not defined, so the "dyn.unload" line must be commented out. Once these are commented out it runs, but with warnings that variables "CPUM" and "CV" are set but not used. CPUM was commented out in the .model file (units conversion can be done in a script). CV was added to the OUTPUTS list, since it may be needed for analysis later.
EXPPULSE (inputs bloc)	Controls the discrete exposure-time profile input via the forcing function.	Model was not producing correct exposure profiles (indicated by time/concentration plots). Was determined to be error in model scripts and not chloroprene.model (new script files have been received from authors of code).
Parameters bloc (non-dynamic)	List and comments of time-independent, body weight-independent unscaled PBPK parameters	No issues (code adequately documented). Note: tissue:blood partition coefficients may differ by up to 1% from the parameters defined in the literature/scripts due to round-off error in the conversion between tissue:air and tissue:blood.
Dynamics bloc		
QC	Cardiac output allometric equation	Equation correct (BW^3/4 scale)
QP	Alveolar ventilation allometric equation	Equation correct (BW^3/4 scale)
QL, QF, QS, QK, QR	Blood flow equations	Equations correct (fractions scaling by total blood flow). Tissue indices match.
QRC	Rapidly Perfused tissues blood flow fraction equation (by difference)	Equation is correct. However, there is no constraint preventing this from being a very small or even negative number for Monte Carlo simulations (if sum of perfused tissue fractions is near or greater than 1 during random draws). Constraint may be in MC scripts.
VL, VLU, VF, VS, VR, VK	Tissue volume equations	Equations correct (fractions scaling by total body weight). Tissue indices match.
ROBC	Rest of body (un-perfused) tissues (by difference)	Equations correct Unclear why this value is necessary, since un-perfused tissues are not modeled. Code states

		it's for "Monte Carlo simulations", but it is only used for a 'vbal' equation (see below). While it does not matter for the model, there is no constraint preventing this from being a negative number (if sum of perfused tissue fractions is near greater than 1 during random draws). Constraint may be in MC scripts.
VMAX, VMAXLU, VMAXKD	Metabolic rates of liver, lung, kidney	Equation correct (BW^3/4 scale)
KFKI, KFLU	Pseudo first-order rates for kidney and lung	Incorrect scaling. These are actually clearance terms, have units of volume/time, hence should be scaled as BW^0.75.
CIX	Ideal gas constant and molecular weight conversion of exposure concentration	Equation is correct (multiplied by MW/24450). Note: It would help to have a comment stating the units conversion (ppm to mg/L)
CI	Inhalation exposure control	Correct (multiplied by binary EXPPULSE switch). Verified by running model to debug prior mistake with forcing function/exposure scripts.
CVx, tissue venous concentrations	Mass of chemical in tissue to venous concentration	Correct (amount/(volume x partition)) for each tissue (tissue/amount/partition indices match).
CPU	Pulmonary mass balance (QP*CI+(QF*CVF + QL*CVL + QS*CVS + QR*CVR + QK*CVK))/(QP/PB+QC)	Equation is correct. Note: An optional addition could be a dead-space fraction, if needed to account for model/data discrepancies for the given QC and QP numbers.
CX	Exhaled concentration = CPU/PB	Equation is correct.
CV	CV = (QF*CVF + QL*CVL + QS*CVS + QR*CVR + QK*CVK)/QC	Correct. Note: this parameter not used in code and was not initially an output. Typically, it is used to condense the pulmonary mass balance equation, or as a biomarker.
CPUM	Units conversion for CPU.	Variable not used in code, and not an output. Unclear of the purpose.
RAI, RAX	Inhalation/exhalation rate equations and mass balance	Equations correct
RAM, RAMLU, RAMK	Rate equations for metabolism in liver, lung, kidney	Correct (reaction concentration is correctly-indexed venous blood concentration for all, using post-BW scaled parameters).
RALU, RAL, RAK, RAS, RAR, RAF	Rate equations for mass balance lung, liver, kidney, slowly perfused, rapidly perfused, fat	Correct. Lung applies CPU (blood concentration at air/blood exchange) input and QC flow. Other systemic organs apply lung venous flow as input, and correctly-indexed venous streams and metabolism as outputs.
Outputs bloc		
MASBAL	MASBAL = AI - AX - (AL+AM+AMLU+ALU+AK+AMK+AS+AR+AF)	Correct (overall mass balance not missing any tissues/sources/sinks)

Cx, tissue concentrations calculations	Concentrations in tissues and for plots	Correct
Dose metrics	Definitions of AMP, AMPLU, AMPK (unit conversions, and cumulative time averaging)	Correct, however, final units should be stated as comments.
Blood/tissue balances	Error checks on total blood and volume fractions	Correct

Physiological parameters and partition coefficients

Physiological parameters for the model are listed in Table S-1 and partition coefficients are listed in Table S-2 of Supp Mat A of the Ramboll (2019) report. All parameters were checked against Brown et al. (1997) or their cited sources and agree with those references, with the following exceptions.

➢ Cardiac output in the mouse (QCC): The value is substantially higher than the value in Brown et al. (1997), but this is addressed in the Ramboll (2019) report. The analysis in the report provides reasonable evidence for use of the alternate value.
➢ Alveolar ventilation (QPC) and cardiac output in humans: the values are substantially higher than those in Brown et al. (1997), but the values in Brown et al. are for individuals at rest. The values come from Clewell et al. (2001) (citation given) and correspond to an average activity over a full work-day.
➢ Volume of other richly perfused tissue (VRC): this should be the sum of tissue fractions for richly perfused tissues *not* included in other compartments of the model. The value includes lung tissue, however, which is a separate compartment. Hence VRC should be reduced to exclude the lung tissue fraction.

Metabolic parameters and IVIVE extrapolation

The following are found in the spreadsheet, EPA Supp Mat D, in the "IVIVE" tab.

➢ **BW values for mice and rats, cells C22-C25**: these differ from the standard BW values listed in table S-1. For the sake of consistency, and since the tissues used to obtain microsomes were likely from juvenile/young adult animals, the lower, standard BW values from Table S-1 should be used here.
➢ **Liver and lung microsome content, cells G24-G26 (rat and human liver) and cells H22-H26 (lung in all species)**: values do not match report text, page 9. The lung values do match Himmelstein et al. (2004b), so the report text could be changed from "20" to "23" to match the spreadsheet, unless the authors believe 20 is correct. Values for rat and human liver may match citations in the spreadsheet (cell G27), but we ask that the authors resolve the discrepancy.
➢ **In Vitro Values of KFLUC for female rat (cell V33) and male rat (cell V38)**: These cells have calculations which are not explained and do not take values from the in vitro metabolic results; e.g., "=1.2/(0.82*2)/1000" in cell V33, which should be just equal to Parameter_Summary cell I18.

Table 2. Quality review of invitro.csl

File or variable	Definition	Notes and determination
	INITIAL bloc	
Model parameters	VMAX1, KM1, RLOSS, VK, P1, A10, VVIAL, VMED, VAIR=VVIAL-VMED, PROT, VINJ	Comments and definitions are poorly documented. **1)** VINJ says "based on Matt email" but the <u>last paragraph</u> of Himmelstein et al. (2004) <u>p. 19</u> gives 400 uL as sample volume for CP oxidation experiments, which differs from 200 uL used for CEO experiments. In V_human.m VINJ is set to 0.0003858 L. An explanation is needed for how VINJ was measured so precisely for humans, and confirmation that it differs from other experiments described in the same paper. Otherwise it should be 0.004 uL for all Himmelstein et al. (2004) data. **2)** Yang et al. (2012), section 2.1.3, states that 200 uL samples were used for those experiments. **3)** VVIAL differs from default (0.01165 L) in the following files: V_kidney.m (0.01163); V_human.m (0.0119573). While the variation likely has minimal impact, a single value should be used in the absence of specific data. Based on report from Matt Himmelstein, a volume of 11.6 mL should be used. Vial volume was determined by adding water and measuring the weight. The SD does not support use of greater accuracy for this value.
Time variables and timing commands	TF, TI, VINJ, TSTOP, POINTS, CINT, TS=TF	Sampling is "disruptive" (in the experiment, sampling the headspace affects the mass balance). The simulated timing should match the experimental condition, but where different replicates used different sample times, a representative

		average would be sufficient (i.e., time of first sample should be average of initial times from replicates). The total number of samples should accurately reflect those taken from each incubation vial.
Initial conditions	CA10=A10/(VAIR+P1*VMED), CM10=CA10*P1, CA1=CA10, CM1=CM10, A1I=0.	Initial conditions would need re-structuring if an alternative 2-compartment model is applied (see below)
DYNAMIC/DERIVATIVE bloc		

Integration and models	Three differential rates (although only a 1-compartment mass balance is performed, which includes a differential loss term) !CD KINETICS (umoles/hr)
	
	R1M=(VMAX1*CM1)/(KM1+CM1)*PROT
	  RRLUNGVK=VK*CM1
	  RRLoss = RLOSS*CM1
	  A1M=INTEG(R1M,0.)
	  ARLUNGVK=INTEG(RRLUNGVK,0.)
	  ARLOSS = INTEG(RRLoss,0.)
	  CA1=(A10-A1M-ARLUNGVK-A1I-ARLOSS)/(VAIR+VMED*P1)
	  CM1=CA1*P1
	  A1=CA1*VAIR+CM1*VMED
		The model assumes instantaneous steady-state in the liquid phase (applying only the media/air partition coefficient for the chemical). Model-predicted headspace concentrations were found to be significantly different if instead applying a more realistic 2-compartment system (assuming concentration-driven mass transport). Estimation of Km would likely be different if model was optimized assuming 2 compartments. Hence, a reasonable estimate for a mass transfer term between liquid gas phase is needed to develop a model that accurately reflects the physical system. Based on example simulations, equilibration must occur in much less than 1 min in order for the assumption to be valid.

DISCRETE bloc		

Discrete events affecting mass balance (doses, sampling, etc).	Contains the routine for mass loss due to sampling
	A1I=A1I+CA1*VINJ
	    SCHEDULE step .AT. TS+TI
	    TS=TS+TI
		See comments under "time variables and timing commands".

Other notes:

VAIR is calculated in the .csl code (VAIR=VVIAL-VMED) based on the CONSTANT values VVIAL and VMED (even if they are not set to defaults). However, this calculation also appears in most of the script (*.m) files. To avoid confusion/redundancy, the line VAIR=VVIAL-VMED should be removed from script files.

Table 3. Check of metabolic parameters (in-vitro) against Yang et al. (2012) and Himmelstein et al. (2004). *[Currently awaiting decisions regarding 2-compartment model]*

File name	Metabolc parameters set	Disp.
V_human.m (PROT=1.0)	VMAX1=0.054; KM1=0.45; VK = 0.0;	
	VMAX1=0.0; KM1=0.0; VK = 0.9/1000;	
	VMAX1=0.405/1000; KM1=0.45; VK = 0;	
V_kidney.m (PROT varies between 2.0 and 3.0 between runs)	VMAX1=0.0027; KM1=0.92; VK = 0.0;	
	VMAX1=0.00226; KM1=0.69; VK = 0;	
	VMAX1=0.00177; KM1=0.37; VK = 0.0;	
	VMAX1=0.0027; KM1=0.69; VK = 0;	
	VMAX1=0.01; KM1=0.5; VK = 0.0;	
	VMAX1=0.01; KM1=0.95; VK = 0;	
	VMAX1=0.00004; KM1=1.7; VK = 0.0;	
	VMAX1=0.0001; KM1=0.95; VK = 0;	
VFM_liver.m (PROT=1)	VMAX1=0.09; KM1=0.53; VK = 0;	
	VMAX1=0.12; KM1=0.95; VK = 0;	
VFM_lung.m (PROT=1)	VMAX1=0.025; KM1=2.78; VK = 0;	
	VMAX1=0.01; KM1=0.95; VK = 0;	
VFR_liver.m (PROT=1)	VMAX1=0.068; KM1=0.82; VK = 0.0;	
	VMAX1=0.055; KM1=0.69; VK = 0;	
VFR_lung.m (PROT=1)	VMAX1=0.0; KM1=0.0; VK = 1.2/1000;	
	VMAX1=1.02/1000; KM1=0.69; VK = 0;	
VMM_liver.m (PROT=1)	VMAX1=0.26; KM1=1.36; VK = 0.0;	
	VMAX1=0.21; KM1=0.95; VK = 0;	
VMM_lung.m (PROT=1)	VMAX1=0.13; KM1=2.0; VK = 0.0;	
	VMAX1=0.05; KM1=0.95; VK = 0;	
VMR_liver.m (PROT=1)	VMAX1=0.077; KM1=0.56; VK = 0.0;	
	VMAX1=0.086; KM1=0.69; VK = 0;	
VMR_lung.m (PROT=1)	VMAX1=0.0; KM1=0.0; VK = 0.9/1000;	
	VMAX1=1.86/1000; KM1=0.69; VK = 0;	

Revised in vitro model (provided July 2019)

Separate m-files were provided which included the in vitro data, but data tables were replicated (perhaps with some rearrangement) in the files that created plots of model simulations vs. the data. This replication is unnecessary and creates the opportunity for discrepancies (QA issues). Hence the duplicate data tables in the plotting scripts were deleted, the scripts now just plot data in arrays defined in the data scripts. Also, system parameters such as the equilibrium partition coefficients and control values that are mostly the same among the experiments (PROT = protein concentration, for example) were moved to a system_params.m script, so they could be checked once and to make it easier to check the values of more experimental-specific parameters in the plotting scripts.

File name	Metabolc parameters set	Disp.
female_mouse_liver.m	VMAX1 was set to 0.11 but listed as 0.108 in Table S-3. In "Posterior Parameters n IVIVE 6 25 2019.xlsx", after changing the number of sig figs shown, 0.108 is confirmed. Changing the value to 0.108 in the script did not significantly impact on the visual plot (on the semi-log scale used). VINJ=0.0002; VVIAL=0.01165; VMAX1=0.108; KM1=0.46; KF=0.0;	VMAX1 set to 0.108
FMouseLiverMCMC1lvl.m	VVIALF, VINJF, and other system parameters set at top of script match; numerical assignments on lines 105-110 also match.	
female_mouse_lung.m	VINJ=0.0002; VVIAL= 0.01165; VMAX1 =0.028; KM1=2.91; KF=0.0;	
FMouse_lung_mcmcrun.m	VVIALF, VINJF, and other system parameters set at top of script match; numerical assignments on lines 107-112 also match.	
female_mouse_kidney.m	VINJ=0.0002; VVIAL=0.01163; VMAX1=0.0; KM1=0.28; KF=0.00043;	
FMouse_KidneyMCMC1lvlvk.m	VVIALF, VINJF, and other system parameters set at top of script match; however, on line 91 it appears VVIAL for females is set to 0.01165, discrepant with value used for other kidney simulations. Impact? Other assignments on lines 92-96 match.	??
male_mouse_liver.m	VINJ=0.0003858; VVIAL=0.0119573; VMAX1=0.23; KM1=0.61; KF=0.0;	
MMouseLiverMCMC1lvl.m	VVIALM, VINJM, and other system parameters set at top of script match; numerical assignments on lines 102-107 also match.	
MMouse_liver_mcmcKG.m	VVIALM, VINJM, and other system parameters set at top of script match; numerical assignments on lines 99-104 also match. I have not checked every line vs. preceding script but it appears to be effective duplicate.	
male_mouse_lung.m	VINJ=0.0003858; VVIAL=0.0119573; VMAX1=0.13; KM1=1.72; KF=0.0;	

MMouse_lung_mcmcrun.m	VVIALM, VINJM, and other system parameters set at top of script match; numerical assignments on lines 97-102 also match.	
male_mouse_kidney.m	VINJ=0.0002; VVIAL=0.01163; VMAX1=0.010; KM1=0.58; KF=0.0;	
MMouseKidneyMCMC1lvl.m	VVIAL, VINJ, and other system parameters set at top of script match; numerical assignments on lines 97-102 also match. ==However, the values of VVIAL and VINJ hard-coded on line 98-99 are 0.0119573 and 0.0003858; i.e., values for male liver and lung experiments. The difference in VINJ in particular is enough to be significant== to kidney Vmax and Km, though impact on PBPK likely to be small.	??
Female_rat_liver.m	VINJ=0.0002; VVIAL= 0.01165; ==VMED=0.002==; VMAX1 =0.072; KM1=0.74; KF=0.0;	
FRatLiverMCMCrun.m	VVIALF, VINJF, and other system parameters set at top of script match; numerical assignments on lines 98-103 also match; ==VMED=0.001 on line 12==. Analysis should be re-run with correct VMED, or value in plot script fixed.	
Female_rat_lung.m	VINJ=0.0002; VVIAL= 0.01165; VMAX1 =0.0; KF=0.00041;	
FRatLungMCMCrun.m	VVIALF, VINJF, and other system parameters set at top of script match; numerical assignments on lines 99-104 also match.	
Female_rat_kidney.m	VINJ=0.0002; VVIAL= 0.01163; ==VMAX1 =0.0036; KM1=0.56==; KF=0.0; values of VMAX1 and KM1 in Table S-3 and 'Posterior Parameters' spreadsheet are 0.0035 and 0.55, respectively. Changing VMAX1 and KM1 to 0.0035 and 0.55 had minimal impact on plots. ==PROT = 1.0==; use of PROT = 2.0 changes simulation results in plot slightly but noticeably.	==VMAX1 and KM1 set to 0.0035 & 0.55, PROT = 2.0.==
FRatKidneyMCMC1lvl.m	VVIAL, VINJ, and other system parameters set at top of script match **except** PROT = 2.0; numerical assignments on lines 98-99 match.	
Male_rat_liver.m	VINJ=0.0003858; VVIAL=0.0119573; VMAX1=0.071; KM1=0.35; KF=0.0;	
MRatLiverMCMCrun.m	VVIALM, VINJM, and other system parameters set at top of script match; numerical assignments on lines 100-105 also match.	
Male_rat_lung.m	VINJ=0.0003858; VVIAL=0.0119573; VMAX1=0.0; KF=0.00087;	
MRatLungMCMCrun.m	VVIALM, VINJM, and other system parameters set at top of script match; numerical assignments on lines 93-98 also match.	
Male_rat_kidney.m	VINJ=0.0002; VVIAL= 0.01163; VMAX1 =0.0041; KM1=0.84; KF=0.0; ==PROT=1.0==; use of PROT = 2.0 changes simulation results in plot slightly but noticeably.	==PROT set to 2.0.==

MRatKidneyMCMC1lvl.m	VVIAL, VINJ, and other system parameters set at top of script match, **except** PROT=2.0; numerical assignments on lines 89-90 match.	
mixed_human_liver.m	VINJ=0.0003858; VVIAL=0.0119573; VMAX1=0.052; KM1=0.32; KF=0.0; if human tissue sampling used VINJ=0.0004 L, this value should be used in script; testing the change had a very slight impact on the simulations as shown in the plot.	
HumanLiverMCMCrun.m	VINJ=0.0004 on line 22; VVIAL and other system parameters match;	??
mixed_human_lung.m	VINJ=0.0004; VVIAL=0.0119573; VMAX1=0.0; KM1=1.0; KF=2.73e-14;	
HumanLungMCMCrun.m	VVIAL, VINJ, and other system parameters set at top of script match; numerical assignments on lines 90-91 match.	